Use of Vascular Assessments and Novel Biomarkers to Predict Cardiovascular Events in Type 2 Diabetes

Use of vascular assessments and novel biomarkers to predict cardiovascular events in type 2 diabetes – the SUMMIT VIP study

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Abstract

Aims: Cardiovascular (CV) disease risk prediction represents an increasing clinical challenge in the treatment of diabetes. We used a panel of vascular imaging, functional assessments and biomarkers reflecting different disease mechanisms to identify clinically useful markers of risk for CV events in subjects with type 2 diabetes mellitus (T2DM) with or without manifest cardiovascular disease (CVD).

Methods and results: The study cohort consisted of 936 subjects with T2DM and a reference population of 487 non-diabetic subjects recruited at four European centers. The three-year CV event rate in subjects with T2DM was higher in those with (n=440) than in those without (n=496) manifest CVD at baseline (5.53 versus 2.15/100 life years, $p<0.0001$). New CV events in T2DM subjects with manifest CVD were associated with higher baseline levels of inflammatory biomarkers (interleukin-6, chemokine ligand 3 and pentraxin 3) and endothelial mitogens (hepatocyte growth factor and vascular endothelial growth factor A), while CV events in T2DM subjects without manifest CVD were associated with more severe baseline atherosclerosis as assessed by carotid ultrasonography. Conventional risk factors, as well as measurements of arterial stiffness and endothelial reactivity, were not associated with CV events. Similar associations with CV events were found in the non-diabetic subjects.

Conclusions: Our observations demonstrate that markers of inflammation and endothelial stress reflects CV risk in T2DM subjects with manifest CVD, while the risk for CV events in T2DM subjects without manifest CVD is primarily related to the severity of atherosclerosis.

Key words: diabetes, cardiovascular events, carotid ultrasound, biomarkers, risk assessment

Diabetes is an important risk factor for cardiovascular disease (CVD) and is associated with a two-fold excess risk of acute myocardial infarction (AMI) and stroke.[1] A recent large Swedish registry study showed that although the incidence of CV events has declined substantially in subjects with diabetes between 1998 and 2014, it still remains significantly higher than in subjects without diabetes.[2] With the worldwide adult prevalence of diabetes rising from 4.7% in 1980 to 8.5% in 2014 the CV complications of diabetes represents a major public health challenge.[3] The increased CV risk associated with diabetes remains essentially the same when adjusting for conventional risk factors.[1] Accordingly, traditional risk score calculators are less useful in diabetes.[4, 5] This has not been a major clinical concern because most guidelines have considered all subjects with diabetes as having high risk based on studies demonstrating that the CV risk is equivalent to non-diabetic subjects with a previous coronary event.[6] However, studies that are more recent have shown that the CV risk in type 2 diabetes mellitus (T2DM) is highly heterogeneous and that many subjects with T2DM have much lower risk of CVD than subjects with established CVD and no diabetes.[7-10] Hence, there is an urgent need to improve CVD risk prediction in T2DM.

The Innovative Medicine Initiative project SUMMIT (SUrrogate markers for Micro- and Macro-vascular hard endpoints for Innovative diabetes Tools) was initiated to novel markers for prediction of CV complications in diabetes. Given the poor risk prediction in diabetics based on traditional CV risk factors alone and the still elusive causes behind the increased CV risk in diabetics, we wanted to assess the ability of a panel of non-invasive vascular imaging, functional vascular tests and emerging biomarkers to predict CV risk in subjects with T2DM. To meet this end, we carried out the SUMMIT Vascular Imaging Prediction (SUMMIT VIP) study. As there is a growing population of T2DM patients with clinically manifest CVD that are at a very high risk for new events[11] we included both subjects with and without prevalent CVD. To determine to what extent our findings would be specific for subjects with T2DM we

also included age and sex-matched non-diabetic subjects with or without prevalent CVD in the study.

Methods are available in the on-line supplement

Results

The baseline investigation of SUMMIT VIP included 458 subjects with T2DM and CVD (myocardial infarction, stroke or lower extremity arterial disease), 527 subjects with T2DM but without clinically manifest CVD and 515 subjects without T2DM (245 with and 270 without CVD). The clinical characteristics of the study cohort have been previously published.[12] Fatal and non-fatal cardiovascular (CV) events were registered during a 3-year follow-up period. Seventy-seven subjects (5.1%) were excluded from the study due to lack of information of clinical events during follow-up. Of the remaining 1423 subjects 154 suffered a cardiovascular event during follow-up (3.6 CV events/100 life years). There were also 23 deaths from non-cardiovascular causes and 9 death of unknown cause (supplemental table 1). Subjects with T2DM and manifest CVD at baseline had a more than two-fold higher CV event rate than those free of CVD at baseline (5.5 versus 2.2/100 life years, $p<0.0001$). In the non-diabetic reference groups, the CV event rate during follow-up was also more than two-fold higher in those with manifest CVD at baseline (4.8 versus 2.0/100 life years, $p=0.002$).

Markers for CV events at follow up in subjects with T2DM and manifest CVD

Occurrence of a new CV event in the T2DM/CVD group was associated with higher baseline HbA1c, but otherwise there were no difference in other conventional risk factors (table 1).

Insulin treatment was more common among those with a new event. However, when including both HbA1c and insulin treatment in a binary logistic regression model together with age, sex, duration of diabetes, smoking, BMI, triglycerides, LDL, HDL and eGFR only HbA1c remained significantly associated with a new CV event (hazard ratio 1.03 (95%CI 1.01-1.03). With the exception of an increased IMT in the left carotid bulb there were no significant differences in carotid IMT, total carotid plaque area, pulse wave velocity, endothelial reactivity or ABPI between those with and without a new CV event (table 2). However, baseline plasma levels of endothelial mitogens and biomarkers reflecting inflammation, such as IL-6 and CCL3, as well as matrix metalloproteinase (MMP)-12, N-terminal prohormone of brain natriuretic peptide (NT-proBNP) and fatty acid binding protein (FABP)-4, were higher in subjects with a new event (table 3). In subjects with T2DM and manifest CVD the discrimination slope of a binary logistic regression model with IL-6 and risk factors (age, sex, duration of diabetes, current smokers, total cholesterol, HDL, HbA1c, systolic blood pressure and ethnicity) was significantly improved by 2.7 percentage points compared with a model without IL-6 (IDI 0.027 [95%CI 0.0064-0.048], p=0.010). The area under the receiver operating characteristic (AUROC) curve was significantly increased with the addition of IL-6 to the risk factor model (AUROC of IL-6 and risk factor model 0.68 [95%CI 0.60-0.75], AUROC of risk factor model 0.60 [95%CI 0.51-0.69, p=0.020). Risk reclassification with the addition of IL-6 to the model was mainly downwards (figure S1 A-B).

Markers for CV events at follow up in T2DM subjects without manifest CVD

There were no significant differences in conventional CV risk factors or medication between those with and without a CV event during follow-up in the T2DM/non-CVD group (table 1).

Those with a CV event had increased IMT in both the left and right bulb, the right common carotid artery (CCA), as well as an increased total carotid plaque area (table 2). Pulse wave velocity, endothelial reactivity and ABPI were not associated with the occurrence of CV events. Subjects with CV events also had higher baseline plasma levels of the apoptosis marker TRAIL receptor 2 and of Growth and Differentiation Factor (GDF)-15, but did not demonstrate the same elevation in endothelial mitogens and inflammatory biomarkers as T2DM subjects with manifest CVD that suffered a new event (table 3). In the T2DM/non-CVD group the discrimination slope of a binary logistic regression model with right CCA IMT and risk factors (age, sex, duration of diabetes, current smokers, total cholesterol, HDL, HbA1c, systolic blood pressure and ethnicity) was significantly improved by 2.4 percentage points compare to a model without IMT (IDI right CCA IMT 0.024 [95%CI 0.0035-0.045]; p=0.022). Risk reclassification with the addition of right CCA IMT to the model was mainly downwards (figure S1 C-D). There was no significant difference in AUROC with the addition of right CCA IMT to the risk factor model (p=0.10).

Markers for CV events at follow up in non-diabetic subjects with manifest CVD

Subjects in the non-diabetic/CVD group that suffered a new event had higher baseline systolic blood pressure and increased IMT in the left carotid bulb, but otherwise there were no differences in conventional risk factors, medication or any of the vascular analyses as compared with those spared from a new event (tables 4 and 5). In line with observations in the T2DM/CVD group, endothelial mitogens and markers of inflammation, as well as MMP-12 and NT-proBNP, were elevated in those with a new CV event (table 6). Tumor necrosis factor (TNF) receptor 1 and Fas, as well as GDF-15, were also elevated in those with a new event.

Markers for CV events at follow up in non-diabetic subjects without manifest CVD

Subjects in the non-diabetic/non-CVD group that suffered a CV event had lower HDL and eGFR as well as higher serum creatinine (table 4). They were also characterized by increased bilateral carotid bulb IMT, right CCA IMT, total carotid plaque area, a lower right ABPI as well as by an increased pulse wave velocity (table 5). Moreover, those with a CV event also had higher plasma levels of IL-6, VEGF A, MMP-7, MMP-12, TNF receptor 1 and GDF-15 (table 6).

Discussion

Using a panel of conventional risk factors, vascular assessments and emerging biomarkers, we demonstrate in the present study that different markers predict risk for CV events in T2DM patients with and without manifest CVD. T2DM subjects with manifest CVD that developed a new event had higher baseline plasma levels of pro-inflammatory cytokines, endothelial mitogens, MMP-12, FABP-4 and the cardiac stress marker NT-proBNP, but were not characterized by more severe atherosclerosis as assessed by carotid IMT (except from a marginally thicker IMT in left carotid bulb) or ABPI. The biological process that results in elevated levels of endothelial mitogens remains to be fully characterized but is likely to involve endothelial stress. Except for a higher HbA1c there were no differences in conventional risk factors between those with and without a new CV event. In general, a similar pattern was observed in the non-diabetic group with established CVD. Subjects with a recurrent event in this group also had higher baseline levels of pro-inflammatory cytokines, endothelial mitogens, MMP-12 and NT-proBNP. They also had higher baseline levels of biomarkers reflecting apoptosis as well as of GDF-15. Both NT-proBNP and GDF15 are established markers of CV risk.[13, 14] Notably, NT-proBNP only predicted CV events in

subjects with established CVD in the present study, irrespective of diabetes status. Other studies have identified elevated NT-proBNP as a CV risk factor in subjects with T2DM,[15] but to our knowledge it has previously not been shown that this primarily is the case for T2DM subjects with prevalent CVD. Subjects without diabetes that suffered a new event had increased carotid bulb IMT at baseline, but there was no difference in CCA IMT, total carotid plaque area or ABPI. Increased arterial stiffness and endothelial dysfunction as assessed by reduced vasodilatation following transient ischemia are well-established vascular complications in diabetes and have been associated with increased CV risk.[16-19] In accordance, subjects with T2DM were found to have increased pulse wave velocity and a lower reactive hyperaemia index at the SUMMIT VIP baseline investigation.[12] In spite of this, neither of these measures predicted the occurrence of a new event in subjects with established CVD in the present study.

Development of a CV event in T2DM subjects without manifest CVD at baseline was associated with increased carotid atherosclerosis as assessed by the CCA and carotid bulb IMT, as well as by increased total carotid plaque area at the baseline investigation. However, biomarkers were less good predictors with only GDF-15 and the apoptosis marker TRAIL receptor 2 being higher in those with a CV event. Moreover, there were no differences in conventional risk factors between those with and without a CV event. In he non-diabetic group without manifest CVD at baseline we found that those that developed a CV event had increased carotid IMT in the right CCA as well as in the left carotid bulb, increased carotid total plaque area, decreased right ABPI, increased pulse wave velocity, as well as higher levels of several known CV risk biomarkers including IL-6, MMP-12 and GDF-15. They also had lower HDL and signs of reduced kidney function. In essence, CV events demonstrated a clear association with established risk factors in this group.

Our observations are in accordance with previous observations that conventional risk factors are poor predictors of CV events in subjects with T2DM, however they suggest some important alternatives. We found that biomarkers reflecting inflammation, as well as endothelial and cardiac stress, are predictors of CV events in subjects with diabetes and manifest CVD, while carotid IMT is a better predictor of risk in diabetic subjects without manifest CVD. Similar, but less distinct associations were observed in non-diabetic subjects with and without manifest CVD at baseline. Increased carotid IMT is a well-established CV risk factor in the general population.[20] In accordance, T2DM subjects with manifest CVD at the baseline investigation had significantly greater carotid IMT than those without manifest CVD.[12] Hence, there seems to be a clear association between atherosclerosis severity and CV risk in subjects with T2DM, but this association diminishes in subjects with manifest CVD. One possible explanation to this could be that a more intense medical intervention in subjects with manifest CVD allows other risk factor mechanisms than those traditionally associated with atherosclerosis progression to become more important as cause of CV events.[21] Hence, biomarkers that associate with CV events in this group could provide information regarding such alternative mechanisms. In the present studies we found that subjects with new events had higher baseline levels of pro-inflammatory biomarkers and endothelial mitogens suggesting the presence of an inflammatory state involving endothelial stress that persist in the presence of statin treatment. In this context it is interesting to note that the recently published Canakinumab Anti-inflammatory Thrombosis Outcome Study (CANTOS) trial showed that IL-1β antibody treatment lowered the rate of recurrent events in patients with history of myocardial infarction and elevated hs-CRP in spite of statin treatment.[22] The mechanisms that maintain vascular inflammation in statin-treated patients remains to fully characterized but may involve factors such as altered shear stress over stenotic plaques, intra-

plaque accumulation of cholesterol crystals, autoimmune responses against modified plaque antigens and chronic infections.[23]

In conclusion, our observations demonstrate that markers of inflammation and endothelial stress are elevated in T2DM subjects with manifest CVD that develop a new event suggesting that these patients may benefit from novel anti-inflammatory CV therapy. The risk for CV events in T2DM subjects without manifest CVD is primarily related to the severity of atherosclerosis.

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Disclosures

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Table 1. Baseline clinical characteristics for subjects with diabetes with or without a cardiovascular event during follow-up

	CVD at baseline (n=440)			**No CVD at baseline (n=496)**		
	No CV event (n=367)	CV event (n=73)	P	No CV event (n=464)	CV event (n=32)	P
Age (years)	69.4±8.5	69.3±8.7	ns	66.5±8.7	68.2±6.1	ns
Sex (% males)	73.4	65.6	ns	62.5	62.5	ns
Current smokers (%)	9.5	16.4	ns	9.1	15.6	ns
Duration of diabetes (years)	12.1±8.6	13.5±8.8	ns	9.1±7.0	11.5±6.3	ns
BMI (kg/m^{-2})	29.9±4.7	30.7±5.6	ns	30.6±5.4	30.4±4.8	ns
Medications						
Insulin (%)	29.3	45.7	0.007	15.8	25.0	ns
Statin (%)	88.9	80.6	(0.05)	61.3	75.0	ns
ACE inhibitors (%)	54.1	44.4	ns	38.5	34.4	ns
Metformin (%)	65.0	61.4	ns	71.4	81.3	ns
Betablockers (%)	57.4	56.9	ns	17.8	9.4	ns
Metabolic factors						
HbA1c (mmol/mmol)	57.7±12.9	62.8±18.7	0.036	56.1±13.6	59.3±12.9	ns
LDL (mmol/L)	2.06±0.77	2.08±0.75	ns	2.41±0.93	2.24±0.76	ns
HDL (mmol/L)	1.20±0.36	1.19±0.33	ns	1.32±0.38	1.30±0.41	ns
Triglycerides (mmol/L)	1.42 (1.02-2.08)	1.45 (1.05-1.84)	ns	1.35 (1.00-1.97)	1.40 (0.90-2.43)	ns
Blood pressure						
Systolic (mmHg)	138±20	140±17	ns	136±18	137±17	ns
Diastolic (mmHg)	76±10	74±9	ns	78±10	77±9	ns
Renal function						
Serum creatinine (μmol/L)	94.1±31.7	97.9±71.2	ns	79.9±20.4	82.5±17.9	ns
eGFR ($mL/min\text{-}^{1}$ per $1.73m^2$)	74.8±26.9	78.0±27.6	ns	85.1±20.7	81.0±20.0	ns

Variables with normal distribution are shown as mean±standard deviation and skewed variables as median and interquartile range. Statistical comparisons between subjects with and without events during follow-up were done using Students´ t-test for variables with normal distribution and with Mann-Whitney U-test for skewed variables.

Table 2. Baseline vascular measurements in subjects with diabetes with or without a cardiovascular event during follow-up

	CVD at baseline (n=440)			**No CVD at baseline (n=496)**		
	No CV event (n=367)	CV event (n=73)	P	No CV event (n=464)	CV event (n=32)	P
CCA IMT, right (mm)	0.97±0.25	0.92±0.20	ns	0.89±0.20	1.00±0.23	0.002
Carotid bulb IMT, right (mm)	1.14 (0.96-1.62)	1.38 (1.01-1.84)	ns	1.03 (0.87-1.24)	1.28 (0.85-1.55)	(0.07)
CCA IMT, left (mm)	0.97±0.25	0.87±0.25	ns	0.92±0.24	1.07±0.49	0.001
Carotid bulb IMT, left (mm)	1.13 (0.95-1.47)	1.27 (1.03-1.67)	0.045	1.05 (0.88-1.27)	1.20 (0.95-1.78)	0.04
Total plaque area (mm^2)	30.4 (15.3-61.4)	36.0 (17.6-68.6)	ns	19.5 (9.5-40.5)	30.4 (16.1-92.2)	0.01
Pulse wave velocity (m/s)	11.8±3.2	11.3±2.3	ns	10.9±2.6	11.6±2.5	ns
Reactive hyperemia index	2.10±0.56	2.16±0.55	ns	2.20±0.65	2.04±0.79	ns
ABPI, right	1.11±0.22	1.05±0.28	(0.07)	1.20±0.15	1.20±0.32	ns
ABPI, left	1.11±0.23	1.10±0.28	ns	1.18±0.28	1.18±0.29	ns

CCA; common carotid artery, IMT; intima-media thickness, ABPI; ankle brachial pressure index. Variables with normal distribution are shown as mean±standard deviation and skewed variables as median and interquartile range. Statistical comparisons between subjects with and without events during follow-up were done using Students´ t-test for variables with normal distribution and with Mann-Whitney U-test for skewed variables.

Table 3. Baseline biomarkers in subjects with diabetes with or without a cardiovascular event during follow-up

	CVD at baseline (n=440)			**No CVD at baseline (n=496)**		
	No CV event (n=367)	CV event (n=73)	P	No CV event (n=464)	CV event (n=32)	P
Inflammation						
IL-6	42.8 (29.8-68.1)	58.5 (42.1-93.5)	0.00005	34.1 (23.8-52.7)	39.5 (24.2-58.0)	ns
CCL3 (MIP-1α)	4.8 (3.9-5.9)	5.1 (4.2-6.7)	0.008	4.6 (3.9-5.9)	4.7 (3.9-5.4)	ns
Pentraxin 3	2.1 (1.7-2.6)	2.3 (2.0-2.7)	0.043	2.1 (1.7-2.6)	2.1 (1.8-2.6)	ns
Endothelial mitogens						
Hepatocyte growth factor	122 (95-148)	134 (107-169)	0.029	110 (88-135)	112 (89-146)	ns
Placental growth factors	189 (153-253)	207 (156-250)	ns	167 (138-204)	184 (143-223)	(0.08)
VEGF A	1520 (1199-1934)	1624 (1246-2131)	0.045	1409 (1136-1783)	1558 (1199-1824)	ns
Matrix proteolysis						
MMP-3	2.6 (2.1-3-5)	2.6 (2.2-3.3)	ns	2.4 (1.9-2.9)	2.2 (2.0-2.6)	ns
MMP-7	517 (333-780)	545 (342-750)	ns	410 (282-580)	539 (347-691)	ns
MMP-12	172 (11-249)	204 (147-289)	0.025	125 (92-180)	130 (102-234)	(0.09)
Apoptosis						
TNF receptor 1	7231 (5743-9153)	7033 (5873-9793)	ns	6295 (5220-7591)	6451 (5433-7899)	ns
TRAIL receptor 2	3.9 (2.7-5.3)	4.2 (2.8-5.4)	ns	3.3 (2.5-4.1)	4.0 (3.1-4.4)	0.039
Fas	231 (186-274)	218 (179-276)	ns	210 (175-247)	212 (169-254)	ns
Other						
NT-proBNP	26.2 (14.3-43.6)	38.6 (20.5-58.9)	0.001	14.3 (9.8-26.0)	16.2 (10.3-22.7)	ns
GDF-15	1458 (1044-2154)	1541 (1143-2073)	ns	1121 (830-1632)	1483 (1180-1898)	0.005
FABP-4	10.7 (7.8-14.9)	13.7 (8.5-19.8)	0.01	9.6 (7.3-12.6)	10.6 (7.6-17.2)	ns

CCL; chemokine ligand 3, MIP1-α; macrophage inflammatory protein 1-α, VEGF A; vascular endothelial growth factor A, MMP; matrix metalloproteinase, TNF; tumor necrosis factor, TRAIL; tumor necrosis factor-related apoptosis-inducing ligand, NT-proBNP; N-terminal prohormone of brain natriuretic peptide, GDF-15; growth differentiation factor-15, FABP-4; fatty acid binding protein-4. All values are arbitrary units shown as median and interquartile range. Statistical comparisons between subjects with and without events during follow-up were done on log2-transformed values using Students´ t-test.

Table 4. Baseline clinical characteristics for non-diabetic controls with or without a cardiovascular event during follow-up

	CVD at baseline (n=234)			**No CVD at baseline (n=253)**		
	No CV event (n=200)	CV event (n=34)	P	No CV event (n=238)	CV event (n=15)	P
Age (years)	69.1±8.4	71.1±7.1	ns	64.6±10.6	70.0±7.2	(0.05)
Sex (% males)	71.5	41.1	ns	55.9	26.7	ns
Current smokers (%)	9.5	14.7	ns	8.9	20.0	ns
BMI (kg/m^{-2})	27.9±4.1	27.5±4.0	ns	26.7±4.0	27.7±2.8	ns
Medications						
Statin (%)	85.5	88.2	ns	21.6	6.7	ns
ACE inhibitors (%)	46.0	32.3	ns	11.1	0	ns
Betablockers (%)	53.0	64.7	ns	10.7	6.7	ns
Metabolic factors						
HbA1c (mmol/mmol)	39.7±4.8	41.2±3.8	ns	38.5±3.9	38.5±4.2	ns
LDL (mmol/L)	2.27±0.76	2.41±0.98	ns	3.18±0.84	3.18±0.73	ns
HDL (mmol/L)	1.36±0.40	1.41±0.41	ns	1.57±0.42	1.29±0.33	0.013
Triglycerides (mmol/L)	1.15 (0.87-1.60)	1.25 (0.89-1.75)	ns	1.20 (0.86-1.60)	1.40 (1.20-2.07)	ns
Blood pressure						
Systolic (mmHg)	132±16	142±24	0.046	132±16	137±18	ns
Diastolic (mmHg)	75±9	76±10	ns	78±9	79±12	ns
Renal function						
Serum creatinine (μmol/L)	86.0±20.2	83.6±26.6	ns	78.3±16.3	90.2±22.5	0.008
eGFR (mL/min^{-1} per $1.73m^2$)	79.1±18.6	77.4±17.1	ns	84.3±15.7	75.7±20.4	0.046

Variables with normal distribution are shown as mean±standard deviation and skewed variables as median and interquartile range. Statistical comparisons between subjects with and without events during follow-up were done using Students´ t-test for variables with normal distribution and with Mann-Whitney U-test for skewed variables.

Table 5. Baseline vascular measurements in subjects without diabetes with or without a cardiovascular event during follow-up

	CVD at baseline (n=234)			**No CVD at baseline (n=253)**		
	No CV event (n=200)	CV event (n=34)	P	No CV event (n=238)	CV event (n=15)	P
CCA IMT, right (mm)	0.90±0.24	0.99±0.28	(0.07)	0.85±0.18	1.07±0.38	0.038
Carotid bulb IMT, right (mm)	1.07 (0.8-1.35)	1.26 (0.95-1.82)	0.047	0.98 (0.85-1.18)	1.08 (0.93-1.49)	ns
CCA IMT, left (mm)	0.92±0.25	0.95±0.21	ns	0.87±0.24	0.99±0.26	(0.07)
Carotid bulb IMT, left (mm)	1.09 (0.93-1.33)	1.37 (1.15-1.63)	0.0003	1.01 (0.84-1.24)	1.29 (0.95-1.53)	0.026
Total plaque area (mm^2)	24.7 (1.1-68.1)	24.2 (15.2-76.9)	ns	18.1 (9.2-41.4)	40.5 (17.7-84.7)	0.014
Pulse wave velocity (m/s)	10.4±2.6	11.1±3.3	ns	9.6±2.4	11.0±2.4	0.045
Reactive hyperemia index	2.29±0.66	2.21±0.55	ns	2.48±0.70	2.18±0.54	ns
ABPI, right	1.14±0.19	1.08±0.20	ns	1.18 ±0.14	1.09 ±0.11	0.019
ABPI, left	1.13±0.18	1.12±0.21	ns	1.18±0.14	1.18±0.11	ns

CCA; common carotid artery, IMT; intima-media thickness, ABPI; ankle brachial pressure index. Variables with normal distribution are shown as mean±standard deviation and skewed variables as median and interquartile range. Statistical comparisons between subjects with and without events during follow-up were done using Students´ t-test for variables with normal distribution and with Mann-Whitney U-test for skewed variabels.

Table 6. Baseline biomarkers in subjects without diabetes with or without a cardiovascular event during follow-up

	CVD at baseline (n=234)			**No CVD at baseline (n=253)**		
	No CV event (n=200)	CV event (n=34)	P	No CV event (n=238)	CV event (n=15)	P
Inflammation						
IL-6	32.4 (22.0-51.3)	42.5 (27.4-69.4)	0.009	29.9 (19.3-47.4)	57.9 (35.4-93-5)	0.0004
CCL3 (MIP1-α)	4.7 (3.7-5.8)	5.9 (4.5-6.7)	0.004	4.6 (3.9-5.6)	5.0 (4.3-6.2)	ns
Pentraxin 3	2.0 (1.7-2.4)	1.9 81.7-2.7)	ns	2.0 (1.7-2.4)	1.9 (1.6-2.39	ns
Endothelial mitogens						
Hepatocyte growth factor	109 (86-133)	122 (101-164)	0.026	99 (79-122)	114 (92-124)	ns
Placental growth factors	177 (145-229)	211 (168-249)	0.02	168 (135-203)	191 (162-239)	(0.07)
VEGF A	1243 (-1031-1641)	1489 (1176-2048)	0.019	1375 (1081-1852)	1624 (1280-1842)	0.042
Matrix proteolysis						
MMP-3	2.5 (1.9-3.2)	2.8 (2.1-3.7)	(0.051)	2.4 (1.9-3.1)	2.8 (2.3-3.2)	ns
MMP-7	431 (309-635)	545 8391-683)	(0.058)	358 (272-506)	580 (366-674)	0.013
MMP-12	145 (105-212)	190 (125-279)	0.026	113 (80-155)	184 (118-277)	0.00005
Apoptosis						
TNF receptor 1	5955 (5008-7383)	6382 (5636-8172)	0.047	5793 (4738-6937)	5957 (5481-8350)	0.039
TRAIL receptor 2	3.1 (2.3-3.9)	3.4 (2.6-5.2)	(0.091)	3.0 (2.2-3.7)	3.3 (2.6-4.9)	ns
Fas	204 (171-244)	226 (195-273)	0.006	195 (164-239)	198 (173-236)	ns
Other						
NT-proBNP	25.8 (15.3-41.4)	42.9 (25.2-63.9)	0.001	17. 0 (10.0-27.1)	20.2 (8.4-26.2)	ns
GDF-15	826 (635-1121)	1176 (816-1424)	0.01	673 (518-887)	875 (630-1420)	0.012
FABP-4	9.0 (7.0-11.5)	9.8 (8.4-13.89	(0.066)	8.6 (6.8-11.2)	9.8 (5.9-14.59	ns

CCL; chemokine ligand 3, MIP1-α; macrophage inflammatory protein 1-α, VEGF A; vascular endothelial growth factor A, MMP; matrix metalloproteinase, TNF; tumor necrosis factor, TRAIL; tumor necrosis factor-related apoptosis-inducing ligand, NT-proBNP; N-terminal prohormone of brain natriuretic peptide, GDF-15; growth differentiation factor-15, FABP-4; fatty acid binding protein-4. All values are arbitrary units shown as median and interquartile range. Statistical comparisons between subjects with and without events during follow-up were done on log2-transformed values using Students´ t-test.